Studies on pollen germination of 40 plant species on sucrose-gelatine and on onion epidermis

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Summary

A rapid and simple method for pollen germination on onion epidermis, suitable for the amateur microscopist and schools as well as in the field or the research laboratory, is described.

Pollen is spread onto the inner surface of onion epidermis supported on a microscope slide, and allowed to germinate in a box lined with damp paper. Pollen germination can be directly assessed on the slide using standard magnification systems. A rapid scoring system for pollen germination is given. Pollen from 40 plant species was investigated for pollen germination. Of the 31 species tested on sucrose-gelatine substrate, 11 showed 11% to 50% pollen germination. Of the 33 species tested on onion epidermis, 15 germinated with high efficiency. There was a strong correlation between ability to show pollen germination on both substrates. Pollen consistently germinated better on onion epidermis than on gelatine substrate. A call for further contributions from amateur microscopists is made.

Introduction

POLLEN GERMINATES ON the stigmas of receptive plants as part of the process of fertilisation. The male nuclei are transported along the growing pollen tube until they reach the female nuclei in the ovules of the recipient plant. This is not the easiest system for observing under the microscope, as the pollen tube is hidden in the female plant tissues, and therefore *in vitro* pollen germination has long held a fascination for microscopists. The material is readily suited to microscopy as little or no dissection is required. *In vitro* pollen germination also provides a useful tool to elicit information on pollen viability, pollen tube growth, and pollen-stigma incompatibility interactions.

Jost [1] gives an excellent introduction to the history of studies on pollen tube germination in the nineteenth century. Shortly after the discovery of the pollen tube on plant stigmas, Mohl [2] mentioned observing pollen tube germination in water with *Morina*. By the middle of the nineteenth century, it was well known that pollen grains germinated in sugar solution [3], leading to detailed studies on optimising sucrose concentrations for a range of species [4, 5, 6]. Later studies demonstrated that pollen germination could be further improved in solution for many species by the presence of small quantities of boron [7, 8] and by the presence of calcium [9, 10].

By far the most critical component in pollen germination, however, is the hydration of the shrunken dehydrated pollen grain. The pollen of most plants is released in a dehydrated state on the anthers, presumably to protect the contents during the transfer from the anthers to a receptive stigma. The pollen has to rehydrate before pollen germination can occur, and a lack or excess of water can have an adverse effect. Whilst prehydration in a humid atmosphere can significantly improve pollen germination in solution, immersion of the pollen is not actually required for germination. Pollen from a range of plant species can be induced to grow pollen tubes in a carefully controlled humid environment [5, 11, 12, 13].

The preparation of precise solutions and defined humidities is simple in the modern laboratory, but there are situations where a simple and safe method to test or demonstrate pollen germination might be preferred, for example in schools without laboratories, out in the field, or at home for the amateur microscopist. This paper describes experiments leading to the fortuitous discovery of a simple system for promoting highly efficient pollen germination in a wide range of species on onion epidermis.

Materials and Methods

[1]. Pollen germination in sucrose solutions

Four millilitre aliquots of solutions of between 5% w/w sucrose (weight for weight sugar to water) and 20% w/w sucrose were prepared using granulated refined sugar from the local supermarket (Tesco, Milton Store) and tap water (Cambridge Water Company, 100 mgL^{-1} – 120 mg L^{-1} Ca+, < 5 mg L^{-1} Boron) in shallow 30 mm wide dishes. Tap water was deliberately chosen as there is a known requirement for calcium for pollen germination [9]. Pollen was transferred into the solutions by rinsing individual stamens from flowers in the liquid. The flowers were picked no more than 1 hour before the experiment. Where possible, different sugar solutions within an experiment were each inoculated with a stamen from the same flower or with stamens

from flowers on the same plant. The dishes containing the inoculated solutions were placed into plastic containers that had been lined with tissue paper soaked in water and had airtight snap-on lids, and were incubated up to 24 h at ambient room temperature. Unless stated otherwise, observations were made 3 to 4 hours after inoculation of the solutions. Pollen tube germination was observed at 40× or 100× magnification with transmitted light.

[2]. Eschscholzia californica *pollen germination on stigma*

Fresh flower heads of *Eschscholzia californica* (Canadian poppy) growing in the author's garden were carefully dissected to isolate stigmas and anthers. Some pollen transfer onto the stigma occurred during this process but no pollen germination was observed at this stage. The stigmas were then incubated either with an additional dusting of pollen on a cavity slide, or with additional pollen in a drop of sucrose solution of either 10% w/w, 15% w/w or 20% w/w sucrose in tap water on a slide and covered with a coverslip. The slides were incubated in the airtight box lined with wet tissue paper for up to 4h.

[3]. Pollen germination on gelatine

Two stock solutions of 8% w/w granulated gelatine (a gift from R Thomas, Germany) with either 10% w/w sucrose or 20% w/w sucrose were prepared with tap water in jars in a boiling water bath. These solutions were then used to prepare aliquots of sucrose gelatine at concentrations of 10%, 12.5%, 15%, 17.5% or 20% sucrose in 8% gelatine. Approximately 1 ml aliquots of the required solutions were cast onto microscope slides, leaving a 1 mm to 2 mm border on three sides of the slide and a 10 mm border at one end to allow handling. The slides were left to set either at ambient room temperature or in the refrigerator for at least 20 minutes and then placed in the plastic air tight box lined with wet tissues until required. Pollen from flowers picked within the hour could then be streaked directly from the anthers onto the sucrose gelatine. Up to six different pollen streaks could be applied to one slide. The slides were then incubated for several hours to overnight in the sealed box. Pollen germination could be observed at 40× and 100× magnification under the microscope, but slides had to be returned to the humid atmosphere of the box to prevent drying out of the pollen tubes.

[4]. Pollen germination on onion epidermis

Eight percent gelatine with or without 15% sucrose was cast on slides as previously. Approximately 1cm square pieces of epidermis were removed from onions and laid onto the gelatine with either their outer surface or their inner surface, which had been in contact with the flesh of the onion segment, facing upwards. Pollen was then dusted from isolated anthers of flowers collected within the hour onto the epidermal surface. The slides were incubated for 3 hours in the sealed box lined with wet tissues. Onion epidermal strips could also be placed directly onto slides pre-moistened by aspiration or with a small drop of water and be inoculated with pollen before incubation in the sealed box.

[5]. Pollen germination on other surfaces

Pollen germination was also tried on glass slides [5, 13], on moist grease-proof paper [1] and on dialysis tubing (exclusion limit 20000 molecular weight) laid on gelatine or on onion epidermis.

[6]. Scoring pollen germination

Pollen grains were considered germinated if the length of the pollen tube exceeded one pollen grain width. Initially, the percentage pollen germination was determined by counting germinated and ungerminated pollen grains over several randomly chosen fields of view. In later experiments, germination was scored visually by scanning the sample and assigning to one of seven score classes as follows:

1 = no germination
2 = 1% to 2% germination
3 = 3% to 10% germination
4 = 11% to 25% germination
5 = 26% to 50% germination
6 = 51% to 90% germination
7 = 91% to 100% germination.

The latter method was fast, reliable, and better suited than direct counting to experiments where a number of samples had to be scored at the same time, or where samples were likely to dry out under prolonged observation.

For photography or observation of pollen tube germination on surfaces, the slide could be covered with a coverslip to prevent drying out on the microscope stage. The light source of the microscope had to be reduced in intensity with neutral density/heat-absorbing filters to prevent

heating of the sample that could locally melt the gelatine solutions within minutes, and cause the accumulation of condensation on the coverslip.

Results

My very first experiment was conducted with pollen from a *Hippeastrum* plant in Germany during late Spring 1999, in a relatively undefined sucrose solution of between 10% and 20% sugar in water. The *Hippeastrum* pollen germinated very well, with 41% of pollen grains germinating. Germination was apparent after 30 minutes and had exceeded 2.5 to 3 pollen grain lengths by 3 hours, reaching 8 pollen grain diameters after 16 hours (overnight). However, when the experiment was repeated at home with pollen from *Eschscholzia californica* (Californian poppy), *Papaver rhoeas* (Field poppy), *Antirrhinum majus* (Snapdragon) and *Passiflora caerulea* (Passionflower) using sucrose solution ranging from 10 to 20% sucrose in water, no germination was observed.

This lack of germination could have been due either to lack of pollen viability, or unfavourable conditions in solution. The viability of *Eschscholzia californica* pollen was studied further as there was a stand of plants available in our garden. *Eschscholzia* pollen dabbed onto isolated stigmas, which were then incubated in a humid atmosphere rather than in solution, showed swelling (hydration) and germination. Pollen grains that were separated from direct contact with the stigma by another pollen grain did hydrate and swell but rarely germinated. *Eschscholzia* pollen also germinated on isolated stigmas in 10% to 20% sugar in water, but pollen grains from the same or from different *Eschscholzia* flowers in the solutions that were not touching the stigma did not show pollen germination over a 24 hour period.

The conclusions were therefore:- [1]. *Eschscholzia californica* pollen could be induced to germinate on stigma surfaces in humid air or in sucrose solution, [2]. sugar solutions did not inhibit pollen germination, [3]. sugar solutions alone were unable to promote pollen germination.

Immersing pollen in sugar solutions may not be the most effective manner of promoting pollen germination [1, 5, 12]. Pollen from a variety of species undergoes limited germination followed by swelling and bursting [1, 12]. This is due to the uptake of sucrose, which in turn results in an increase in water uptake into the pollen grain until the grain bursts. Other species have a demonstrated requirement for a more limited availability of water [1, 5, 12]. One method of achieving a more restricted hydration is by germination on solid substrates such as gelatine in a humid atmosphere [5, 12, 13, 14]. Accordingly, pollen from 31 species was applied to a substrate of 8% gelatine, 15% sucrose in water.

Sixteen of the 31 species tried showed pollen germination, of which 11 showed 11% to 50% of pollen grains germinating (table 1), including *Eschscholzia californica*, *Antirrhinum majus*, *Papaver rhoeas* and *Lotus subbiflorus*. Repeated experiments with these plants showed a variability in pollen germination. To establish whether this could be genetic or experimental, a more detailed experiment was carried out with *Lotus subbiflorus* rather than with *Eschscholzia californica*, as the latter were all concentrated in one stand in the garden and originated from one seed lot. *Lotus subbiflorus* grew in isolated patches on the lawns and verges of the Cambridge Science Park and it was felt that flowers from distinct patches would differ genetically.

Eight flowerheads from individual clumps of *Lotus subbiflorus* separated by at least 1 meter were collected (UK Ordnance Survey grid reference TL 468 617). Six flowerheads were randomly chosen and used to create streaks on five separate gelatine-sucrose slides. Only three of the six flowers were found to be at the correct stage to give a pollen streak . The percentage germination of each of five pollen streaks per flower was counted and the results plotted in figure 1. Germination rates across replicates varied by up to 15% for each flower, showing that there was considerable variation in the determination of percentage germination for a given sample of *Lotus subbiflorus* pollen. This was consistent with the observations made with *Eschscholzia* and *Antirrhinum* and also indicated that the assignment of pollen germination results into score classes instead of using absolute percentage germination was valid. Whilst the pollen from *Lotus subbiflorus* plant 1 appeared to germinate better than that from plants 2 & 3, the differences between plants or slides were not statistically significant in a Chi square test.

A large proportion of pollen grains were still bursting on the gelatine. In order to control water uptake by the grains even further, germination was tested on grease-proof paper [1] with *Eschscholzia*, dialysis membrane and on onion epidermis with *Eschscholzia*, *Antirrhinum* and *Rubus* pollen. Onion epidermis was chosen fortuitously

on the basis of its being a membrane system readily available to the amateur.

Pollen did not appear to germinate on the grease-proof paper, and visibility was poor on this substrate with top or transmitted lighting. Limiting hydration of pollen on the remaining substrates had two effects, both reducing bursting of pollen grains and affecting the germination rate. Figure 2 shows that the percentage of pollen grains bursting was reduced from above 40% for *Eschscholzia* and *Antirrhinum* on gelatine to 20% or less on dialysis membrane. On the onion epidermis, the percentage of bursting pollen grains was reduced even further. Figure 3 shows that few pollen grains on dialysis membrane germi-

Table 1. Pollen germination for 41 plant species on gelatine or onion epidermis.

Family	Latin name	Common name	sug+gel	onion ep
Caprifoliaceae	*Lonicera sp.*	Honeysuckle	5	7
Crassulaceae	*Sedum sp.*	Sedum		7
Guttiferae	*Hypericum calycinum*	Rose of Sharon	4	7
Leguminosae	*Lotus subbiflorus*	Hairy birdsfoot trefoil	4	7
Loganiacea	*Buddlea davidii*	Buddlea		7
Papaveraceae	*Papaver rhoeas*	Field poppy	4	7
Papaveraceae	*Eschscholzia californica*	Californian poppy	5	7
Ranunculaceae	*Nigella damascena*	Love in a mist		7
Rosaceae	*Potentilla astrosanguinea*	Potentilla	4	7
Rosaceae	*Rubus fruticum*	Bramble	5	7
Scrophulariaceae	*Antirrhinum majus*	Snapdragon	4	7
Scrophulariaceae	*Diascia sp.*	Diascia	4	7
Compositae	*Heiracium sp.*	Hawkweed sp.	1	6
Iridaceae	*Crocosmia*			5
Ranunculaceae	*Ranunculus repens*	Buttercup	2	5
Rosaceae	*Fragraria vesca*	Wild strawberry	2	4
Caryophyllaceae	*Dianthus sp.*	Carnation		3
Guttiferae	*Hypericum perforatum*	Perforate St Johns wort		2
Tiliaceae	*Tilia x europaea*	Lime	1	2
Balsaminaceae	*Impatiens wallerina*	Busy Lizzie	1	1
Compositae	*Dimorphotheca sp.*	Osteospermum		1
Compositae	*Hypochaeris radicata*	Common Catsear	1	1
Compositae	*Tripleurospermum iodorum*	Scentless mayweed	1	1
Compositae	*Leucanthemum superbum*	Shasta daisy	1	1
Compositae	*Bellis perennis*	Daisy	2	1
Convulvulaceae	*Calystegia sepium*	Bindweed		1
Geraniaceae	*Geranium pyrenaicum*	Hedgerow cranesbill	1	1
Geraniaceae	*Geranium grandiflorum*	Geranium		1
Gesneriaceae	*Sainpaulia ionanthe*	African violet	1	1
Malvaceae	*Malva alcea*	Mallow	1	1
Onagraceae	*Fuchsia sp.*	Fuchsia	1	1
Passifloraceae	*Passiflora caerulea*	Passionflower	1	1
Urticaceae	*Urtica dioica*	Nettle		1
Convulvulaceae	*Convolvulus arvensis*	Field bindweed	3	
Cornaceae	*Cornus Alba*	Dogwood	4	
Myrtaceae	*Eucalyptus langsdownea*		2	
Resedaceae	*Reseda lutea*	Weld	4	
Rosaceae	*Rosa sp.*	Rose	1	
Compositae	*Taraxacum vulgare*	Dandelion	1	
Compositae	*Senecia jacobaea*	Ragwort	1	
Compositae	*Achillea millefolium*	Yarrow	1	

Sug + gel = solid 8% gelatine, 15% sucrose as a substrate for pollen germination. Onion ep = the inner face of an onion epidermal strip as a substrate for pollen germination. Numbers indicate score classes for pollen germination, where 1 = no germination, 2 = 1% to 2% germination, 3 = 3% to 10% germination, 4 = 11% to 25% germination, 5 = 26% to 50% germination, 6 = 51% to 90% germination, 7 = 91% to 100% germination.

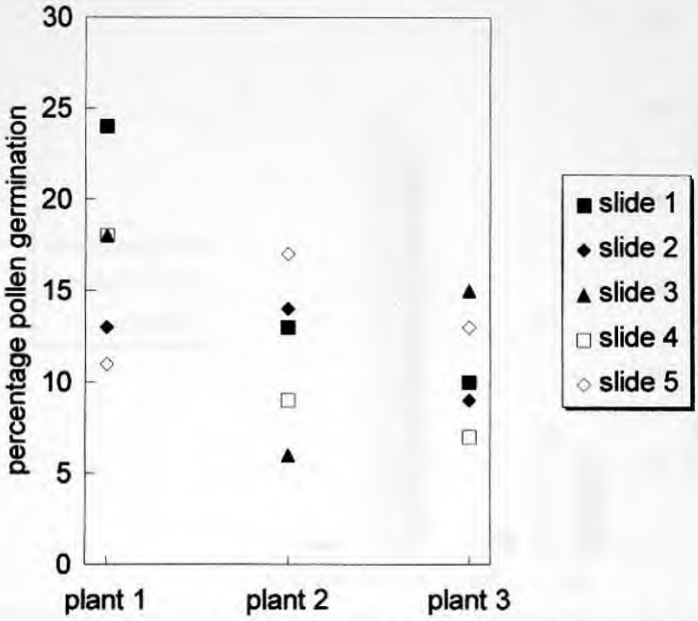


FIG. 1. Variation in pollen germination between three flowers of *Lotus subbiflorus* and between five replicate pollen germinations for each flower.

Data presented as the percentage pollen germination observed on five separate slides of 8% gelatine, 15% sucrose substrate.

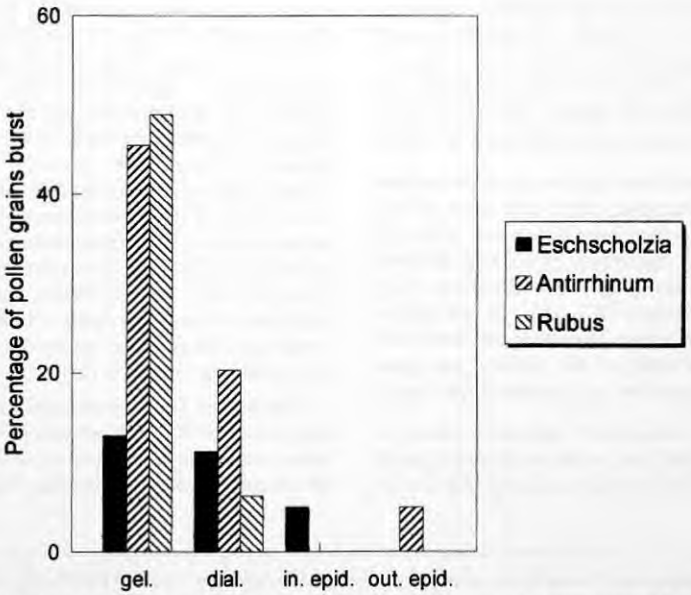


FIG. 2. Percentage of pollen grains bursting on four different solid substrates for three species, *Eschscholzia californica*, *Antirrhinum majus* and *Rubus fruticum*.

Gel = 8% gelatine, 15% sucrose. Dial = dialysis tubing on 8% gelatine, 15% sucrose. In epid = inner surface of onion epidermis. Out. epid. = outer surface of onion epidermis.

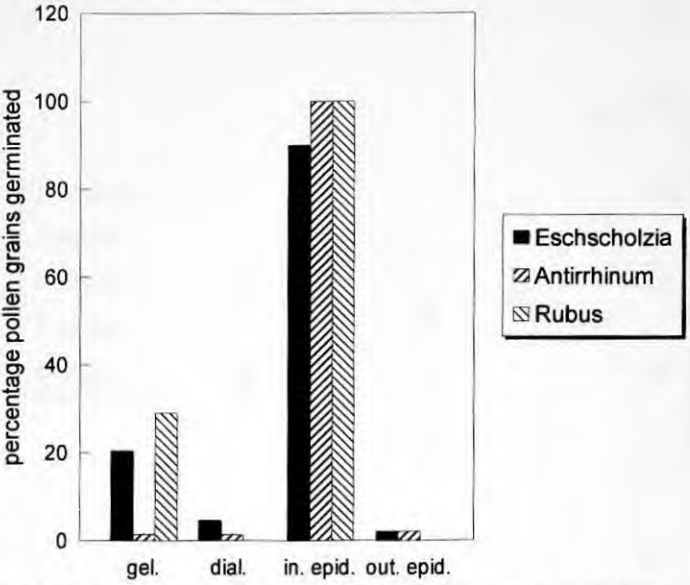


FIG. 3. Percentage of pollen grains germinating on four different solid substrates for three species, *Eschscholzia californica*, *Antirrhinum majus* and *Rubus fruticum*.

Gel = 8% gelatine, 15% sucrose. Dial = dialysis tubing on 8% gelatine, 15% sucrose. In epid = inner surface of onion epidermis. Out. epid. = outer surface of onion epidermis.

nated (1% to 5%) compared to pollen on gelatine alone. However, there was a dramatic increase to almost 100% germination with all three species for the pollen placed on the onion epidermis but only on the epidermal surface that had been in contact with the onion tissue (figures 3 & 4 & 5).

As can be seen from figure 4, an added advantage to the microscopist was that the onion epidermis supporting pollen germination was relatively transparent and featureless, permitting observation with transmitted light with objectives of up to 40× magnification (NA 0.65). As the pollen grains and germinating tubes were not immersed in liquid, the contrast of the subjects was good enough for observations on unstained specimens.

Germination was equally high with onion epidermis on gelatine, and onion epidermis directly on the slide with or without a cover slip. In the absence of a cover slip, the *Eschscholzia* and *Antirrhinum* pollen showed a significant proportion of pollen tubes growing into the air above the onion skin, a phenomenon also observed by Molisch [5]. The epidermis for the initial experiments had been obtained from large brown onions. A comparison between large brown onions, large white onions, and red onions purchased from Tesco demonstrated that all could promote almost 100% germination of *Eschscholzia* pollen. John Garrett [15] was also able to demonstrate this effect with purchased onions and with epidermal strips from leeks. However, Garrett found that a local crop of onions was unable to promote pollen germination [15].

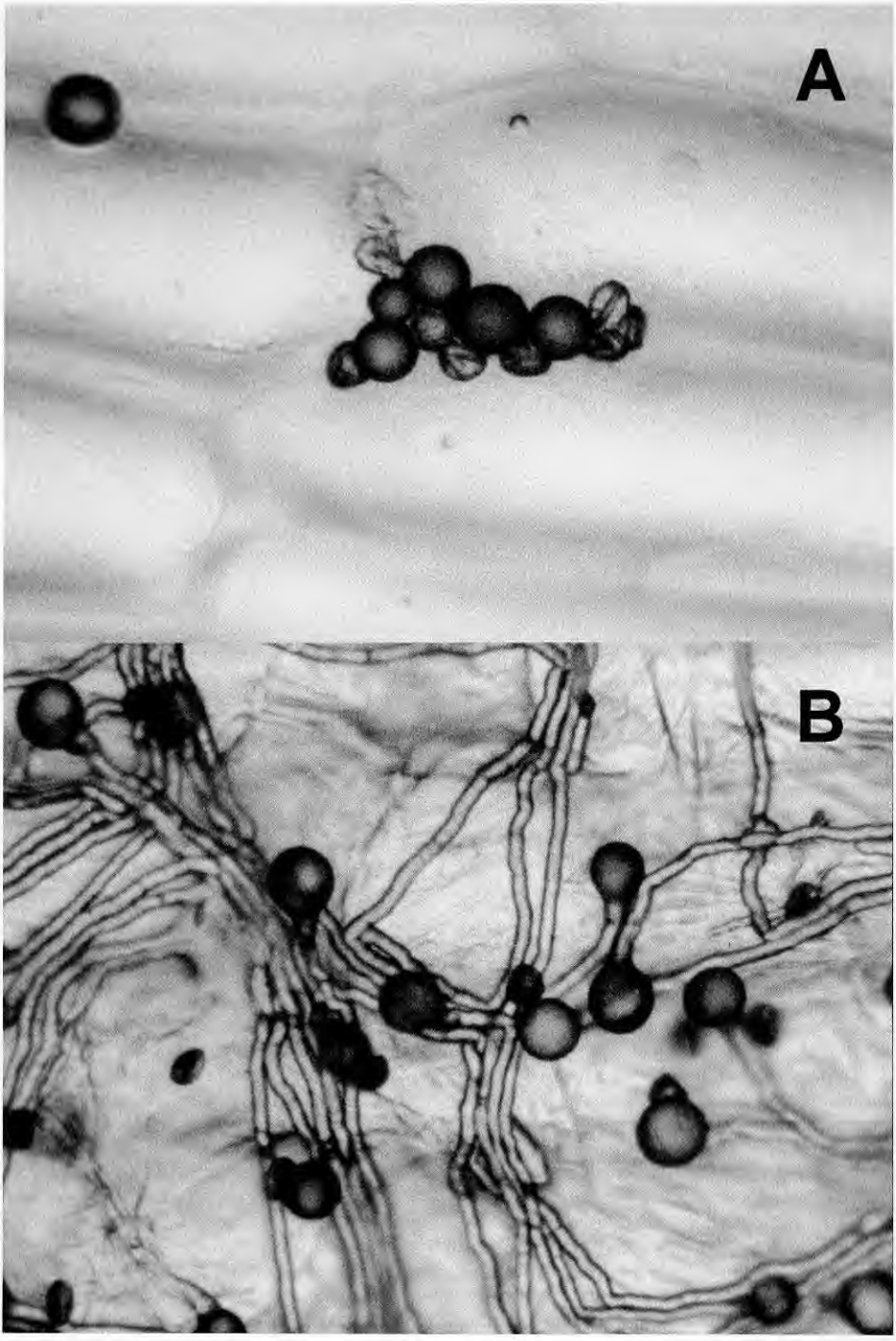
Rinsing the Tesco onion epidermis under running water for 10 to 20 seconds prior to a germination experiment had no effect, whilst rinsing for 60 seconds reduced germination slightly to 51%–

FIG. 4. Pollen germination of *Eschscholzia californica* on onion epidermal strips, recorded three hours after application.

A = pollen on the outer surface of an onion epidermal strip.

B = pollen on the inner surface of an onion epidermal strip.

Photographs were taken with a 35 mm film colour negative camera using a 10× objective and 10× eyepiece. Positive, high resolution Kodak digital images were obtained and edited for this article.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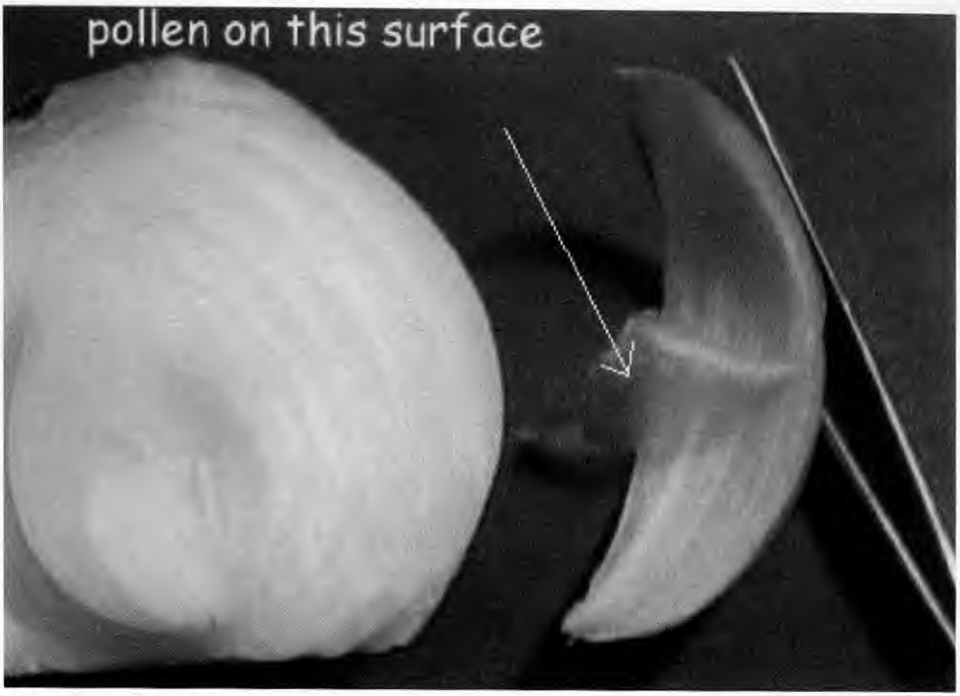

FIG. 5. Photo showing preferred surface of onion epidermal strip for pollen germination experiments.
Digital image courtesy of John Garrett, Ipswich who has also successfully demonstrated pollen germination on onion epidermis using the method developed here.

90% (score 6). If pollen was placed on dialysis tubing on top of onion epidermis, then germination scores of between 2 and 5 (2% to 50%) could be achieved. The surface of an onion segment with the epidermis removed could also promote pollen germination. However, onion sap from the segment smeared on a slide and air dried supported pollen hydration in a humid chamber but did not promote germination.

Pollen germination on the inner surface of onion epidermis was tested for 33 species (table 1). Of these, 15 germinated with a high efficiency whilst 14 species did not show pollen germination. There is a strong correlation visible in table 1 between the efficiency of pollen germination on sucrose-gelatine and that on the inner onion epidermal surface. Five of the non-germinating species were Compositae which have been shown by others to be difficult to germinate *in vitro* [1]. It was observed that amongst the non-germinating species discussed in this paper, several were notable for their pollen depositing a large 'footprint' of material shed from the pollen coat, e.g. *Geranium pyrenaicum*, *G. grandiflorum*, *Malva alcea*, *Passiflora caerulea* and *Saintpaulia ionanthe*.

Discussion

From a learning point of view as an amateur microscopist, my experience with the different methods for stimulating pollen tube germination reiterated historical findings. Germination in sucrose solutions alone only worked with a small number of species, and was fraught by the need to find and optimise the addition of a range of other components not readily available, controllable, or suited to the amateur. Germination on gelatine surfaces was more successful but still relatively low, suggesting that controlling hydration of the pollen might provide the key. However, controlling hydration precisely would require a much more controlled environment than used here, e.g. the use of smaller chambers, strict temperature control and solutions of defined hygroscopicity, as demonstrated by Walderdorf [12]. The success with the inner face of onion epidermal strips was a totally fortuitous discovery!

Germination on onion epidermis provides a halfway house between an *in vivo* and *in vitro* system. Similar approaches had been tried at the turn of the last century. At that period, scientists had tried using fruit compotes [5] or the leaves of aquatic plants with some success [1]. The added advantage of using onion epidermis over previously published methods is its transparency to light, which permits observation of the pollen germination with transmitted light on most microscopes. The success of the onion epidermis method is probably provided by a combination of controlled hydration afforded by the inner epidermal surface, and the provision of a wide range of experimentally undefined components at near physiological concentrations, released from damaged cells by the act of removing the epidermal strip. These are likely to include sugars, calcium [8,9], boron ions [7] and the lipids recently described by Wolters-Arts *et al* [16].

It is significant that although pollen from a range of species germinated well on the onion epidermis, the same species had previously shown a predisposition to germinate on the gelatine sugar surface. Other species which are known to be difficult such as the Compositae still remain recalcitrant. Unfortunately, the experiments with onion epidermis were conducted later in the summer after most grasses had shed their pollen. Grass pollens have been shown to have stricter hydration requirements but can be made to germinate *in vivo* [1] and could form the subject of future studies with onion epidermis. Of the other non-germinating species, it was noted that they had a noticeable 'footprint' of shed material that had been carried on the pollen coat. With such species, it is possible that there is a more complex interaction in terms of available factors or communication between pollen and stigma, leading to a tighter control of pollen germination [10, 17, 18]. Again, this may be a direction for future work on germination on onion epidermis.

Despite the limitations mentioned above, a significant number of species are likely to show pollen germination on onion epidermis. This means that pollen germination experiments can easily, safely and cheaply be set up by most people with access to a magnifying system of 40× magnification or more, i.e. schools, basic research facilities and of course, the amateur microscopist. A number of readers may wish to conduct their own research and publish their findings separately. However, to those who would like to take part in a larger project, get some credit and yet be spared the stress of compiling an article, I invite you to conduct your experiments according to some basic principles set out below and send me the results to collate, hopefully for publication in this journal!

Standardised pollen germination and recording

a. Remove an approximately 1 cm square piece of onion epidermis.

b. Breathe on or slightly dampen a microscope slide and lay the onion epidermal square on the slide with the face that had been in contact with the flesh of the onion segment upwards (see figure 5).

c. Either use immediately or place in sealed box lined with damp tissue.

d. Apply pollen to the surface of the onion epidermis within 1 hour of picking the flower. Place in humid sealed container.

e. Score for germination 3 hours after applying the pollen to the epidermis. View using a ×10 objective (circa 100 × total magnification). Pollen grains are considered germinated if the length of the pollen tube exceeds one pollen grain width. Germination is scored visually by scanning the sample and assigning to one of seven score classes as follows:

   1 = no germination
   2 = 1% to 2% germination
   3 = 3% to 10% germination
   4 = 11% to 25% germination
   5 = 26% to 50% germination
   6 = 51% to 90% germination
   7 = 91% to 100% germination

f. Send the following information to the author:

   1. Your name (optional)
   2. Where geographically you obtained the pollen (flower). Latitude & Longitude if possible.
   3. Date you conducted experiment.
   4. Local time of day experiment result observed (24 hour clock or state am or pm.).
   5. Name of flower from which you took pollen – Latin name and common name if possible.
   6. Any additional points you thought of interest.
   7. Please try to conduct three separate experi-

ments with the same species to obtain three separate pollen germination scores. Record each result separately and do not worry if there is variation.

NOTE: Once you find a species that gives very good germination, always include a test slide with that species with any new species you are testing. That way you can be sure that any results you see are consistent and real. For photography or observation of pollen tube germination, the slide can be covered with a coverslip to prevent drying out on the microscope stage. The light source of the microscope has to be reduced with neutral density/heat-absorbing filters (or use a cold light source such as a white LED [15] to prevent heating of the sample that could cause the accumulation of condensation on the coverslip].

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